UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: EPA's Response to Comments Received on the September 08, 2017, Proposed Registration Decision for the New Active Ingredient 89668-U, *Wolbachia pipientis,* ZAP strain in male *Aedes albopictus* (Asian tiger mosquitoes) - FIFRA (Docket ID Number: EPA-HQ-OPP-2016-0205; FRL- XXXX-XX)

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BACKGROUND

On February 5, 2016, the U.S. Environmental Protection Agency (EPA or the Agency) received an application for registration of a microbial pesticide product containing the new active ingredient *Wolbachia pipientis*, ZAP strain (also referred to as *w*Pip strain; ZAP Males®) for use in *Aedes albopictus* from MosquitoMate, Inc. (MosquitoMate; EPA File Symbol 89668-U).

After completion of the full application review, on September 12, 2017, EPA posted to www.regulations.gov the proposed registration decision together with the final scientific risk assessments and draft labeling, and opened a 15-day public comment period on the proposed action in accordance with Agency policy (Docket No. EPA-HQ-OPP-2016-0205; available at www.regulations.gov). Concurrently, the Agency posted its response to the public comments received on the Notice of Receipt (NOR) for the registration application of this new active ingredient in the same Docket (NOR published in the *Federal Register* of April 28, 2016 (81 FR 25401)). In response to these publications, EPA received ten public comments from private citizens, a company (Oxitec, Ltd.), two non-governmental organizations (Beyond Pesticides and Center for Food Safety), and three professional associations (Entomological Society of America, the American Mosquito Control Association, and the Western Integrated Pest Management Center). EPA appreciates all of the comments received.

PUBLIC COMMENTS AND EPA'S RESPONSE

EPA grouped comments that highlighted similar topics and generated one response to each grouping. When grouping text from multiple sources, EPA provides specific details as to where the text originates. Where appropriate, EPA refers to its response to comments received on the NOR for this new active ingredient for a more detailed discussion (EPA-HQ-OPP-2016-0205-0022, available at www.regulations.gov).

I. HOMEOWNER USE

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec, Ltd.) - "Finally, we note that MosquitoMate included with its registration application a request that ZAP mosquitoes be approved for sale and distribution to homeowners for domestic use. The record does not to any extent support such use, and EPA should under no circumstances approve this product for homeowner use. EPA's science reviews and decision documents make clear that proper use of ZAP mosquitoes is complex, and involves significant preparatory and monitoring work. No ordinary homeowner is equipped to carry out these preparatory and monitoring functions. The draft label must be revised so that it cannot be interpreted as permitting homeowner use of ZAP mosquitoes.

[…]

Third, sales and distribution of ZAP mosquitoes to homeowners for domestic use should not be permitted under any circumstances. The ZAP mosquito label should be revised to make clear that homeowner use is not intended or permitted.

[...]

Document EPA-HQ-OPP-2016-205-14, *BPPD's review of efficacy data on male releases of Ae. albopictus infected with ZAP strain Wolbachia to support homeowner use* indicates that the applicant also requested registration of ZAP mosquitoes for homeowner use. EPA has not included a proposed label for homeowner use in the public docket, nor has EPA indicated an intent to register the product for homeowner use. Information included in the public docket does not support registration of ZAP mosquitoes for homeowner use. Moreover, EPA's science reviews include numerous use requirements for ZAP mosquitoes that are wholly incompatible with sale and distribution to untrained homeowners for domestic use.

Document EPA-HQ-OPP-2016-205-21, *Proposed Registration Decision for the New Active Ingredient Wolbachia pipientis ZAP (wPip) strain in Aedes albopictus* states that "ZAP Males must be released in excess of wild males (10 ZAP Males per 1 wild male), and these ratios must be empirically determined by the applicator for each geographic area throughout the months of the mosquito season, including its peak season." Well, this determination is, of course, beyond the means of homeowners. Determinations of this sort must be conducted by vector control professionals - not by ordinary homeowners. Document EPA-HQ-OPP-2016-205-17, *Amended BPPD review of efficacy data on male releases of Ae. albopictus infected with ZAP strain Wolbachia pipientis and modeling analyses for population dynamics of Ae. albopictus* recommends that "a *Wolbachia* ZAP male release program be made part of an integrated vector management (IVM) program" that is a "multi-tactic strategy consisting of e.g., larvicide and adulticide applications in addition to ZAP male releases with considerations given to appropriate timing of each application." Conduct of an IVM program in concert with proper releases of ZAP mosquitoes is beyond the capability of homeowners.

Also, permitting homeowner use may significantly increase the risk that ZAP mosquitoes may be intentionally released in States where such releases are not approved. This may be a particular concern in unapproved areas bordering States where the registration is approved, e.g., Virginia, South Carolina, North Carolina, Georgia, Alabama, Mississippi, Oklahoma, and Arkansas. These concerns will be entirely eliminated if use of ZAP mosquitoes is restricted to vector control and public health bodies.

Finally, we note that the proposed label permits use by "persons under direct contract with MosquitoMate, Inc., for the purpose of application of this pesticide." EPA must make clear that this language is not intended, and cannot be used, to permit MosquitoMate to contract directly with homeowners to permit use of the product by such homeowners on individual properties. Given the express recommendations of EPA's science reviewers regarding proper use of ZAP mosquitoes, and the proposed use requirements in EPA's proposed decision, it must be made unequivocally clear that this product is only to be used by trained professionals.

There is no basis in the public record that supports registration of ZAP mosquitoes for domestic homeowner use. EPA should not, under any circumstances, approve ZAP mosquitoes for homeowner use. EPA's science reviews and decision documents make clear that proper use of ZAP mosquitoes is complex, and involves significant preparatory and monitoring work. No ordinary homeowner is equipped to carry out these preparatory and monitoring functions."

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "BPPD's review of efficacy data on male releases of Ae. albopictus infected with ZAP strain Wolbachia to support homeowner use (EPA-HQ-OPP-2016-0205-0014): The one small study examined by the BPPD on homeowner use of the ZAP strain males does not provide enough data to support the efficacy of having homeowners release the mosquitoes. The mosquitoes should be released only by a mosquito control district or a mosquito control professional company."

EPA's Response

Upon request from the applicant, EPA explored the possibility for homeowner use in the context of single-point releases, *i.e.*, on a single property as opposed to wide-area applications, as part of the scientific review of the product efficacy data. The results of this review are summarized in "BPPD's review of efficacy data on male releases of *Ae. albopictus* infected with ZAP strain *Wolbachia* to support homeowner use," dated February 02, 2017 (EPA-HQ-OPP-2016-0205-0014). The Agency concluded therein that, based on current data, it is likely not feasible for the average homeowner to overcome the technical challenges in order to comply with the label instructions and thus with the Federal law (Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 12(a)(2)(G)). Consequently, the ZAP-infected males (ZAP Males®) are only proposed for use by trained mosquito control professionals and no homeowner use-specific label was made available for public comment.

A separate result of this review was that, while efficacy of the ZAP Males® was shown to be reduced when applied on a single property compared to wide-area applications, it remained sufficient to suppress *Aedes albopictus* populations in the treated areas. Therefore, use of ZAP Males® on single properties is proposed as part of its use when applied by trained applicators in accordance with the label instructions for single property releases (see Sub-label A, draft label; EPA-HQ-OPP-2016-0205-0015).

II. MOSQUITO GENDER SEPARATION PROCESS

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec, Ltd.) - "The materials released to the docket on September 12 and September 19 do not adequately address the issue of how the production methods proposed by the applicant MosquitoMate will ensure that ZAP females are not released to the environment. All that is apparent from the materials released to the docket are EPA's conclusions that the applicant has in place production methods to limit release of ZAP females, but there is not any information that explains what those conclusions are based on, or that the public may comment on. Oxitec requests that EPA make available the actual information on ZAP mosquito production methods that will enable informed comment on the Agency's conclusions.

[…]

Oxitec has serious concerns regarding specific aspects of the proposed ZAP mosquito registration decision and the draft registration materials. First, the bases of the recommendations and conclusions regarding gender sorting are not apparent. Because of significant concern

regarding whether the asserted gender sorting can be accomplished at production scale, the registration must include mandatory monitoring for released ZAP females."

[…]

MosquitoMate quotes a female release rate of 1:250,000 males, based on size sorting pupae, and then visual inspection of adults from very small scale releases performed in 2013 and 2015. At small scale this may be achievable, but at the scale of production proposed in the Section 3 application, to cover entire States, this is unrealistic and unprecedented. We are unaware of any data or information posted that support a conclusion that the necessary female sorting can be accomplished at that scale of production. Moreover, without any available quality control procedures reported or able to be confirmed, one cannot adequately address the potential deficiencies in the MosquitoMate production protocols."

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "We agree that the current procedures for separating male from female mosquitoes appear to be sufficient. However, given that MosquitoMate is partnering with Google's Alphabet/Verily to use its Verily robotic processes to transfer the Wolbaccia [sic] to male mosquitoes (MIT Technology Review, July 14, 2017), it seems prudent to require a future assessment of the efficiency of the robotic process. There is mention of a "mechanical" sorting device at 3.1.2 (see page 9 of 17 in EPA HQ OPP-2016-0205-002), but no discussion of the Verily robotic device now being used in a partnership between MosquitoMate and the Google owned robotics company. If the mechanical device is not the Verily robot, then a review of the efficacy of the robotic gender separation must be performed prior to the approval of these mosquitoes for wide use."

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "Further, while male *Aedes albopictus* are to be released, there is a possibility of the unintended release of infected females. The registrant estimates there may be a female contamination factor of one female per 250,000 males (EPA-HQ-OPP-2016-0205-0018), but since visual checks are to be employed to identify female contamination, we can expect this number to be much higher."

EPA's Response

Gender separation of mosquitoes prior to release is accomplished by a two-step process in which ZAP-infected pupae in the laboratory are first separated via a mechanical sorting device and the resulting batch is subsequently visually inspected for the presence of females (EPA-HQ-OPP-2016-0205-0018). Mechanical sorting of *Aedes albopictus* at this life stage is possible because male and female pupae differ in size. EPA determined that data submitted by MosquitoMate satisfactorily demonstrate that this process yields the indicated 1: 250,000 ratio of ZAP-infected females to ZAP Males® and made the determination that this ratio is adequate to support a FIFRA section 3 registration.

After considering the public comments, EPA imposed additional terms on the registration that require MosquitoMate to monitor for ZAP-infected females in the environment and confirm a gender ratio of no more than 1 ZAP-infected female to 250,000 ZAP-infected males. Should MosquitoMate wish to change the currently approved manufacturing process in the future, the company will have to provide a new protocol to satisfy the requirements set forth in OPPTS

guideline 885.1200 (Microbial Pesticide Test Guidelines; Manufacturing Process). Before any new manufacturing process can be implemented, EPA will review the new procedures for their adequacy.

On the request to make available the "actual information on ZAP mosquito production methods," a Freedom of Information Act (FOIA) request may be submitted to EPA for the release of such information. However, certain data submitted to the Agency as part of the pesticide registration application are protected from disclosure under FIFRA sections 10(b) and 10(g) (Limitations on Disclosure of Information under Pesticide Law). To the extent permitted, EPA discussed the manufacturing process of the ZAP Males® in its scientific reviews, the proposed registration decision document, the final registration, and the response to comments received on the NOR (EPA-HQ-OPP-2016-0205-0018; EPA-HQ-OPP-2016-0205-0021; EPA-HQ-OPP-2016-0205-0022).

III. POTENTIAL FOR ESTABLISHMENT OF THE ZAP STRAIN IN WILD *AEDES ALBOPICTUS* POPULATIONS

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - "Female ZAP mosquitoes that are released will carry the ZAP *Wolbachia* strain in their eggs, and such offspring will survive to adulthood. The female offspring from such eggs will also inherit the ZAP *Wolbachia* strain and their offspring can survive, whether they mate a ZAP male or a wild-type male. In typical Malthusian dynamics, this will lead to the ZAP strain spreading throughout an *Aedes albopictus* population.

[…]

The most likely failure of the ZAP *Aedes albopictus* control program is through the release of females resulting in replacement of the wild *albopictus* population with mosquitoes carrying the ZAP *Wolbachia* strain. If this occurs the ZAP mosquito product can no longer be efficacious, and the ZAP *Wolbachia* strain, which is not naturally found in *Aedes albopictus,* could potentially spread throughout the United States. Moreover, in such circumstances, the spread of ZAP *Wolbachia* mosquitoes would not necessarily be limited to the States where the registration may be approved."

EPA's Response

While it is correct that based on the *Wolbachia* biology, female ZAP-infected mosquitoes can successfully mate with both ZAP, and non-ZAP-infected males and that the resulting offspring would mature into adulthood and themselves carry the ZAP strain, the establishment of ZAP-infected *Aedes albopictus* depends on the potential release of ZAP-infected females into the environment. This was previously discussed in greater detail in the response to comments to the NOR and the proposed registration decision (EPA-HQ-OPP-2016-0205-0022 and EPA-HQ-OPP-2016-0205-0021). At this time, the Agency does not have any information to support the commenter's assertion that the expected release-rate of ZAP-infected females (1:250,000) will

result in the establishment of the ZAP strain in a given population, that it would do so in an exponential manner, or that it would result in its establishment in *Aedes albopictus* populations throughout the United States, as claimed by the commenter. On the contrary, based on experiments conducted in the field, introduction of a new *Wolbachia* strain into an entire mosquito population appears to be challenging in a real world setting, even when there is an intentional release of *Wolbachia*-carrying females (World Mosquito Program, formerly Eliminate Dengue Program). As outlined in section "II. Mosquito Gender Separation Process," above, as part of the terms of the registration, MosquitoMate must monitor for the presence of released ZAP-infected females in the environment, which will facilitate early detection of the bacterium in wild *Aedes albopictus* populations, if it were to be present.

IV. POTENTIAL FOR ADVERSE EFFECTS ON HUMAN HEALTH

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - "In addition, were there to be widespread dissemination of ZAP *Wolbachia* mosquitoes, the human health risks that were proposed to be mitigated by the 'negligible risk' exposure of ZAP females would have to be completely re-evaluated. As stated in EPA's *Human Health Assessment* (EPA-HQ-OPP-2016-0205-18), "the evaluation of the unintended release of females is the critical factor for the purposes of exposure assessment.

[...]

In typical Malthusian dynamics, this will lead to the ZAP strain spreading throughout an *Aedes albopictus* population. When this occurs, the ZAP males are no longer effective and this leads to a failure of control. In addition, and of significant concern, humans, pets, and wild animals (including endangered animals), which are all targets of the *Aedes albopictus* mosquito species, will then be exposed to the ZAP *Wolbachia* from bites by infected females.

EPA has assessed the chances of population replacement through the release of ZAP females as negligible. However, the assessment of risk to humans and animals changes dramatically if ZAP mosquitoes become established in any area.

There are two critical processes that must be evaluated to mitigate this risk:

1) The ZAP mosquito application claims 1 in 250,000 sorting efficiency. Therefore, any registration of ZAP mosquito should require quality control procedures for every batch of ZAP males produced to ensure that ZAP females are not released. If there are more than 1 female in every 250,000 males it should be reported to the EPA. If this occurs the releases should be stopped and the sorting process assessed and corrected.

2) The establishment of the ZAP *Wolbachia* strain in the wild. This can be assessed through BG sentinel traps and the testing of female *Aedes albopictus* for the presence of the ZAP *Wolbachia* strain. If female *Aedes albopictus* are found with the ZAP strain, all releases of ZAP mosquitoes must be halted and monitoring for females continued for at least 8 weeks to determine if the ZAP strain has established in the wild. If establishment of the

ZAP strain is confirmed, through the detection of more females infected with the ZAP strain, the registration should be immediately cancelled and no further releases should be allowed."

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "The Human Health Assessment (EPA-HQ-OPP-2016-0205-0018) only examines whether the MosquitoMate ZAP mosquito can transmit arboviruses as it is being presently raised. Given that there will be considerable variability in how conditions are established in a dozen states where the mosquitoes will be bred in the future, regular testing for arboviruses such as West Nile, known to be present in these states, should be a part of future trials. It is noted that the blood that female mosquitoes are fed during the rearing process is tested for the arbovirus vesicular stomatitis. This needs to be expanded to include testing for all arboviruses known to be in the area."

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "Female mosquitoes bite and infect their human host with viruses, and whether there is a human risk from being exposed to *W. pipientis* is not known or has been considered.

[...]

EPA believes the gender separation technique employed by the registrant to be "highly efficient" and that there will be "negligible exposure" to infected females and subsequent human health risk (EPA-HQ-OPP-2016-0205-0021). We believe a thorough human health assessment for *W. pipientis* is still warranted and must be conducted.

[...]

However, we urge EPA to remain vigilant and use caution with this new technology, conduct a full human health assessment, and consider the potential for resistance and ecological impacts."

EPA's Response

Regarding the potential for ZAP Males® to vector pathogens of concern to human health, the ZAP-infected mosquitoes are reared in a laboratory, which limits their exposure to human viruses before they are released into the environment. Even so, MosquitoMate's manufacturing process does include periodic testing of these populations for the presence of the following viruses that are of importance to human health: Zika, Chikungunya, West Nile, Dengue, and Equine Encephalitis. Testing is also conducted for vesicular stomatitis, a disease that primarily manifests in cattle and horses. For a more detailed discussion on this topic, please see EPA-HQ-OPP-2016-0205-0021 Proposed Registration Decision and EPA-HQ-OPP-2016-0205-0018 Human Health Assessment.

Based on the periodic testing of the mosquito population for viruses that are of human health concern, the fact that male mosquitoes are not vectors of human pathogens, and the negligible risk for ZAP-infected female releases (EPA-HQ-OPP-2016-0205-0018), the Agency concludes that the risk of exposure to the *Wolbachia* ZAP strain is negligible and thus poses a negligible human health risk. Further, section "II. Mosquito Gender Separation Process," above, outlines additional terms of the registration to confirm that unacceptable numbers of female mosquitoes are not released.

In response to the comments referencing "future trials," EPA is issuing a pesticide registration under FIFRA section 3 and not an Experimental Use Permit (EUP) under FIFRA section 5. Thus, the registration of this product will allow MosquitoMate to sell and distribute the ZAP Males® within the specified boundaries for the duration of five years without the requirement for further trials (EPA-HQ-OPP-2016-0205-0015 Product label and EPA-HQ-OPP-2016-0021 Proposed registration decision).

V. POTENTIAL FOR ADVERSE EFFECTS ON THE ENVIRONMENT

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - "Moreover, as Oxitec has detailed in previous comments submitted on the record in this matter, the potential for release of *Wolbachia pipientis,* ZAP strain (ZAP) females could potentially result in serious and significant adverse environmental effects.

[…]

Discovery of an established population of ZAP mosquitoes would constitute a serious adverse environmental effect;

[…]

When this occurs [the spreading of the ZAP strain throughout an *Aedes albopictus* population], the ZAP males are no longer effective and this leads to a failure of control. In addition, and of significant concern, humans, pets, and wild animals (including endangered animals), which are all targets of the *Aedes albopictus* mosquito species, will then be exposed to the ZAP *Wolbachia* from bites by infected females."

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "(i) ensure complete testing of the potential range of adverse biological effects (A naturally occurring bacterium that is known to have several different effects on an insect's reproductive system and is considered a "symbiont" certainly has possibilities for coevolving with its host in ways that benefit the mosquito and the bacterium.)

[…]

The registrant, MosquitoMate Inc., has submitted a section 3 registration petition, including the ZAP strain Wolbachia-infected male mosquitoes for the product, ZAP Males®. Under the *Federal Insecticide Fungicide and Rodenticide Act* (FIFRA), EPA has a responsibility to ensure that this product does not pose unreasonable adverse effects to the environment. In EPA's response document to comments received in 2016 (EPA-HQ-OPP-2016-0205-0022) several commenters raised valid concerns we believe the agency has to address. There is concern for potential non-target ecological effects where Wolbachia may affect insects in the environment by changing behavior, disease transmission, gene expression and biology. More information is needed on any unintended ecological impacts."

From EPA-HQ-OPP-2016-0205-XXXX (Anonymous) - "Please do not approve! More research needs to be done on the biological and ecological effects the release of these mosquitoes will have on every ecosystem that they will be released into. Just one example is how they will effect

[sic] struggling bat and fish populations that rely on mosquitoes and there [sic] larva for there [sic] main food source!"

EPA's Response

With the information presently available to EPA, the Agency determined that the ZAP Males® will not pose adverse effects on the environment, and refers to the response to comments document on the NOR of this registration application for a detailed discussion on these topics (EPA-HQ-OPP-2016-0205-0022). The commenters did not provide new information to support the claims that any of the mechanisms outlined above would lead to adverse environmental effects.

EPA addressed environmental concerns over accidental female release in its response to comments on the NOR for this registration action (see pages 14-15 of EPA-HQ-OPP-2016-0205). The response included a discussion of the methods to be used to ensure that females are accurately sorted from males, and an analysis of the potential for a population of mosquitoes with the *Wolbachia* ZAP strain to become established. EPA concluded that the probability of accidental female release is low, and that establishment of a population of these mosquitoes in the event of an accidental release is very unlikely. Further, section "II. Mosquito Gender Separation Process," above, outlines additional terms of the registration to confirm that unacceptable numbers of female mosquitoes are not released. EPA also addressed effects on nontarget organisms in the ecological risk assessment for *Wolbachia pipientis* ZAP strain, including issues raised in the comments above (see EPA-HQ-OPP-2016-0205-0019 and pages 15-18 of EPA-HQ-OPP-2016-0205). EPA concluded that adverse effects to nontarget organisms are unlikely. The commenters do not provide any substantive information beyond what has already been considered that would alter these analyses or EPA's conclusions.

VI. PESTICIDE RESISTANCE DEVELOPMENT

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) – "When this [ZAP strain spreading throughout an *Aedes albopictus* population] occurs, the ZAP males are no longer effective and this leads to a failure of control."

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "There are questions around the use of naturally-occurring bacterium to suppress insect populations and the onset of resistance. This has been seen previously with Bacillus thuringiensis (Bt) in other species. Does the agency have a plan to mitigate the onset of resistance?

[…]

However, we urge EPA to remain vigilant and use caution with this new technology, conduct a full human health assessment, and consider the potential for resistance and ecological impacts."

EPA's Response

After considering the public comments on the potential for resistance development, the Agency required MosquitoMate to monitor for ZAP-infected females in the environment as part of an insect resistance management (IRM) program. In the event of resistance development, the goal of this proactive monitoring strategy will be to enable the detection of resistance at an early stage and increase the chance to preserve the effectiveness of the ZAP Males®. These types of monitoring programs are analogous to those required as part of the IRM strategies for plant-incorporated protectants (PIPs), including those containing *Bt* proteins. Under FIFRA Section 6(a)(2), registrants are required to inform the Agency if, at any time after the registration of a pesticide, the registrant has any additional factual information regarding unreasonable adverse effects on the environment of the pesticide. Per 40 CFR § 159.188(c), such incidents include development of pesticide resistance. These reports are then used to make decisions on the appropriate response to mitigate any adverse effects, including the development of a specific resistance management plan, which may include measures such as notification to stakeholders and the prohibition of pesticide sales in certain geographic locations.

VII. ZAP MALES® IN THE CONTEXT OF INTEGRATED VECTOR MANAGEMENT

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "4. Clarify whether the suggestion of "IVM-integrated vector management" in the amended efficacy review (EPA-HQ-OPP-2016-0205-0017) is a requirement of the approval:

The BPPD notes that, "A Wolbachia ZAP male release program for Ae. albopictus has the potential to reduce the female mosquito density over time and, therefore, can provide societal benefits. BPPD concludes that MosquitoMate should combine their ZAP male releases with an integrated vector management (IVM) approach (e.g., use sequentially with larvacide [sic] and/or adulticide treatments) in order to increase their efficacy (or % reduction in females) and to generate more consistent results. As an IVM tool, the proposed product would also provide environmental benefits because it could result in a reduction of chemical pesticide applications for mosquito control." Is this a requirement for further trials and registration of MosquitoMate's products or is this just a suggestion?"

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "Will these mosquitoes be applied to areas that will continue adulticiding activities, thereby eliminating *W. pipientis*-infected mosquitoes from the area and reducing efficacy?"

EPA's Response

The numerical efficacy listed on the ZAP Males® draft label was achieved without the use of mosquitocides. The reported product performance levels were determined to be sufficient to support the registration of the product without the requirement for the concurrent use of integrated vector management (IVM) techniques, such as adulticiding or larviciding, when it is used in accordance with the label. However, under circumstances in which control of mosquito species other than *Aedes albopictus* or stronger reduction in *Aedes albopictus* populations is

desired, the Agency encourages the use of adulticides and larvicides in addition to the ZAP Males®.

As described on the label, care must be taken when applying adulticides in areas in which live mosquitoes are being released to avoid harming the ZAP Males®. The label of the adulticide must be consulted prior to utilizing ZAP Males® in an IVM program that includes adulticiding to time the applications appropriately. On the other hand, efficacy of the ZAP Males® is not affected by the application of larvicides, as these do not interfere with the mating process or affect the ZAP Males® directly.

VIII. EFFICACY OF THE ZAP MALES®

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Beyond Pesticides) - "However, there are still questions regarding the use of this new product that we believe should prompt the agency to take a precautionary approach. There is little independent data on the overall efficacy of the *Wolbachia pipientis* ZAP strain, and we therefore do not know how effective the release of significant numbers of *W. pipientis*-infected mosquitoes will have (sic) on competing with wild males and successfully breeding with females. How many females would need to encounter an infected male to render a sufficient reduction in healthy larvae? Will these mosquitoes be applied to areas that will continue adulticiding activities, thereby eliminating *W. pipientis*-infected mosquitoes from the area and reducing efficacy?"

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - "The most likely failure of the ZAP *Aedes albopictus* control program is through the release of females resulting in replacement of the wild *albopictus* population with mosquitoes carrying the ZAP *Wolbachia* strain. If this occurs the ZAP mosquito product can no longer be efficacious, and the ZAP *Wolbachia* strain, which is not naturally found in *Aedes albopictus,* could potentially spread throughout the United States. Moreover, in such circumstances, the spread of ZAP *Wolbachia* mosquitoes would not necessarily be limited to the States where the registration may be approved."

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "4. Clarify whether the suggestion of "IVM-integrated vector management" in the amended efficacy review (EPA-HQ-OPP-2016-0205-0017) is a requirement of the approval:"

[…]

"Conversely, the BPPD notes, 'In future trials, MosquitoMate needs to assure that 1) control and treated sites are separated by approximately 800 m, 2) baseline (a priori) monitoring of mosquito pressures occurs in the presence of uniformly applied mosquito abatement, and 3) treated and control sites occur in the same general suburb with similar characteristics (e.g. socio economics, human density, mosquito abatement, etc.).' This reads like a requirement."

EPA's Response

In their registration application, MosquitoMate provided empirical data on the efficacy of the product for both wide-area applications and single-point releases. As the ZAP Males® have to overwhelm the wild male *Aedes albopictus* population in order to be effective, the ratio at which this is achieved was tested and determined in the field during the preceding EUPs (EPA Reg. No. 89668-EUP-1). These data were evaluated by EPA and found to demonstrate sufficient performance to support the registration of this product when used in accordance with the label (EPA-HQ-OPP-2016-0205-0015). As discussed in section "VII. ZAP Males® in the Context of Integrated Vector Management," above, the efficacy of the ZAP Males® is not expected to be adversely affected if adulticiding is timed appropriately.

EPA recognizes that the presence of ZAP-infected females in the environment could be indicative of early resistance development. By requiring monitoring of ZAP-infected females in the environment as a term of the registration (see sections "II. Mosquito Gender Separation Process" and "VI. Pesticide Resistance Development," above), it is ensured that the company will be able to detect the onset of resistance to the *Wolbachia* ZAP strain in a timely manner.

Commenters requested clarification on EPA's statements on the design of future trials. At this time, these are recommendations for future submissions of similar types of products and are not terms for the registration of the ZAP Males®.

In response to the comment on the lack of independent data, it should be noted that some of the information furnished to EPA by MosquitoMate is also published in the peer-reviewed scientific literature that is available to the public (Mains *et al.*, 2016, discussed in the efficacy review for use by homeowners EPA-HQ-OPP-2016-0205-0014).

IX. USER MANUAL

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - "The proposed registration decision and draft User Manual are either inconsistent in significant respects with the findings and recommendations of EPA's science reviews, or lack sufficient clarity to demonstrate how certain recommendations will be addressed.

[...]

Second, MosquitoMate's so-called User Manual is unclear, confusing, and out of date. It should not be publicly released until it is corrected and substantially revised.

[...]

The ZAP Mosquito Draft User Manual

Oxitec finds the draft "User Manual" to be confusing, misleading, and out of date. EPA should require a complete re-write of this document, starting with a determination as to what is its intended purpose. Is this document intended to be distributed to public health and vector control specialists? If so, it is likely to be of no benefit or utility to such professionals at all. To the extent that such professionals would need education on *Aedes albopictus* (which is unlikely),

more reliable and useful information may be easily obtained from the Centers for Disease Control website. If the document is intended to inform the public, as currently drafted, it fails that purpose.

Oxitec respectfully suggests that the User Manual not be approved for any use related to the proposed ZAP mosquito registration. To the extent that the User Manual is allowed to be distributed, EPA must require the following corrections and revisions:

The User Manual must incorporate the 2017 CDC *Aedes albopictus* range map, not the outdated 2016 range map.

The User Manual must explicitly state the limited geographic scope of the registration, so that readers are not confused as to where the product may lawfully be used.

The User Manual should explicitly state that release of ZAP mosquitoes in States other than those expressly identified on the label is a violation of Federal law.

Consistent with BPPD's Geographic Restriction memorandum, the User Manual should note that not all of the 21 States included on the label that have climatic conditions similar to the States where efficacy testing was conducted actually have *Aedes albopictus* populations

Consistent with BPPD's efficacy review, the User Manual must accurately state the typical and maximum extent of *Aedes albopictus* dispersal"

EPA's Response

For the purposes of this registration decision, EPA evaluated the ZAP Males® user manual, which is considered to be labeling, for false or misleading claims. A pesticide is misbranded, and therefore cannot be lawfully sold or distributed (FIFRA section 12(a)(1)(E)), if, *inter alia*, its labeling bears any statement, design or graphic representation that is false or misleading (FIFRA section 2(q)(1)(A)). 40 CFR § 156.10(a)(5) provides examples of statements that constitute misbranding. EPA found no false or misleading statements in the user manual. However, after further consideration, EPA determined that the product label alone provides adequate guidance to the user on the application of the pesticide and the user manual will not be provided or made available, either at the time the product is purchased or online. However, the company has the option to submit a manual for labelling review to EPA at a later time.

X. TERMS AND CONDITIONS OF THE REGISTRATION

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Oxitec Ltd.) - Moreover, if monitoring of any releases of ZAP mosquitoes reveals that female ZAP mosquitoes have been released, the registration should include a term and condition mandating that EPA immediately be informed of such release and that the ZAP mosquito registration be suspended immediately and releases halted until a full investigation has been conducted to determine the circumstances that led to release of

ZAP females. If female ZAP mosquitoes are released and the ZAP strain is found in wild type mosquitoes, EPA must immediately initiate cancellation proceedings for the registration.

[…]

Registration Suspension and Cancellation
It is imperative that EPA impose mandatory registration suspension requirements in the event that ZAP female mosquitoes are detected in the environment

If post-release monitoring detects the presence of ZAP females, all releases must be halted and the ZAP registration immediately suspended. EPA should include a term and condition of registration that, if ZAP *Wolbachia* females are detected in the environment, the registration will be suspended under FIFRA Section 6(c); if the ZAP strain is found to have become established in the wild, EPA will initiate cancellation proceedings under FIFRA Section 6. Discovery of an established population of ZAP mosquitoes would constitute a serious adverse environmental effect; therefore, the ZAP registration must be immediately cancelled and action on any pending registration applications immediately suspended, as the ZAP strain would no longer be effective at controlling *Aedes albopictus* and additional releases could result in this non-wild type strain becoming more established in the environment.

[…]

Post-Release Monitoring
It is imperative that EPA impose mandatory post-release monitoring requirements that will ensure the detection of any ZAP female mosquitoes in the environment. For the reasons set forth above, Oxitec requests that EPA include an explicit term and condition of registration requiring post-release monitoring of *Aedes albopictus* populations to determine if any ZAP females have been released.

EPA's Response

After having considered the public comments on the potential for resistance development, the Agency is requiring MosquitoMate to monitor for ZAP-infected females in the environment, which increases the success of any mitigation measures. The registrant has a statutory obligation to inform the Agency if, at any time after the registration of the pesticide, the registrant has any additional factual information regarding unreasonable adverse effects on the environment (see section "VI. Pesticide Resistance Development," above). This is true for microbial and conventional pesticides alike. These adverse effects reports are then evaluated by the Agency and appropriate measures are proposed on a case by case basis.

XI. PROPOSED GEOGRAPHICAL RESTRICTIONS

Comments

From EPA-HQ-OPP-2016-0205-XXXX (Center for Food Safety) - "2. Geographical range of the trial is inappropriate: Geographic restriction is one of the standards for biosafety review. In short, geography can serve to limit the spread of the new organism. The rationale used to determine in which states this five year trial would occur is inadequate. The report states: "BPPD

[Biopesticides and Pollution Prevention Division] identified that efficacy data generated in more northern climate regions of the U.S. cannot be extrapolated to infer expected efficacy of this product in southern U.S. climate regions, which are considered high mosquito population pressure areas for Ae. albopictus." However, the proposed states for release include several states (TN, MD, DC, DE and KY) where all or a significant part of the state is situated in the south U.S. climatically. Even though Kentucky was one of the states where the early trials were conducted, the area of the state around the Mississippi River is very "southern" in climate and should be excluded from future trials. A better "mapping" of where this large trial could take place would assess the climatic conditions on a county, not state basis. Or alternatively, a standard source, such as the USDA plant hardiness zones, could be used a proxy for areas where hard freeze might prevent year around mosquito breeding (USDA Plant Hardiness Zone Map). Relying on state boundaries alone is insufficient. Either find a better method of selecting states for the trial, or exclude TN, MD, DC, DE and western KY. Moreover, much of California, especially the Central Valley and most of Southern California, should be considered "southern" areas, where there is a high likelihood of the mosquitoes breeding throughout the year.

EPA's response

MosquitoMate applied for a registration of the *Wolbachia* ZAP strain under FIFRA section 3. In its evaluation of the efficacy data, the Agency determined that the geographic locations in which the trials were conducted during the EUPs were not sufficient to support a registration in States other than those 20 and the District of Columbia listed in the proposed decision document (EPA File Symbol 89668-EUP-1; EPA-HQ-OPP-2016-0205-0021). Rather, the Agency decided to register the ZAP Males® only in States that are climatically similar to those for which efficacy data were presented. The Agency utilized the U.S. standard regions for temperature and precipitation, first described in Karl and Koss, 1984, to determine such similarity. These 9 climate regions represent areas that are similar in temperature and precipitation, which are two variables that are of significance to the biology of the mosquito. The regions were formed based on over 80 years of climate data. Because local microclimates within a given area or between two consecutive years can be more or less conducive to the thriving of the mosquitoes, a standard was determined that ensures the efficacy of the product for the duration of the registration. One of these standards is the ratio at which the ZAP Males® must be released, which will account for seasonal and annual variations in mosquito populations. The other standard relies on the history of climate data, which provides confidence that the average climate in each of the States is sufficiently similar that efficacy of the product remains ensured. A detailed discussion of the reasons for this geographic restriction was provided in the scientific rationale for the proposed geographic restriction for the FIFRA Section (3) *Wolbachia pipientis* ZAP strain (*Aedes albopictus*) registration application. Reg. No. 89668-U (B-Clade *Wolbachia pipientis*); PC Code: 069035; dated March 22, 2017; EPA-HQ-OPP-2016-0205-0013).

XII. REFERENCES

EPA-HQ-OPP-2016-0205-0013: Scientific rationale for the proposed geographic restriction for the FIFRA Section (3) Wolbachia pipientis ZAP strain (Aedes albopictus) registration application. Reg. No. 89668-U (B-Clade Wolbachia pipientis); PC Code: 069035. Dated March 02, 2017.

EPA-HQ-OPP-2016-0205-0014: BPPD's review of efficacy data on male releases of *Ae. albopictus* infected with ZAP strain *Wolbachia* to support homeowner use. Dated February 02, 2017.

EPA-HQ-OPP-2016-0205-0015: Draft Product Label *Wolbachia pipientis* ZAP strain - ZAP Males®.

EPA-HQ-OPP-2016-0205-0016: ZAP Males® User Manual.

EPA-HQ-OPP-2016-0205-0017: Amended BPPD review of efficacy data on male releases of *Ae. albopictus* infected with ZAP strain Wolbachia pipientis and modeling analyses for population dynamics of *Ae. albopictus* (MRIDs 496802-01, 497831-01, and 498307-03; EPA Reg. No. 89668-U) (B-Clade *Wolbachia pipientis*; PC Code: 069035). Dated November 30, 2016.

EPA-HQ-OPP-2016-0205-0018: Human Health Assessment, Review of the MosquitoMate Inc., Updated Manufacturing Process, ZAP strain Origin Validation, and Sex Separation data for the Section 3 Registration of the ZAP strain *Wolbachia pipientis* in *Aedes albopictus.* Dated July 24, 2017.

EPA-HQ-OPP-2016-0205-0019: Revised Ecological Risk Assessment for the Section 3 registration of the microbial pesticide end-use product ZAP mosquito larvae: PC Code: 069035; EPA File Symbol 89668-U; Decision No. 513757; Submission Nos. 980717, 985153; DP Barcode No: 432413, 433706. MRID Nos: 49530604, 49830704-06. Dated June 06, 2017.

EPA-HQ-OPP-2016-0205-0021: Proposed Registration Decision for the New Active Ingredient *Wolbachia pipientis* ZAP (*w*Pip) strain in *Aedes albopictus*. Dated September 08, 2017.

EPA-HQ-OPP-2016-0205-0022 (supersedes EPA-HQ-OPP-2016-0205-0020): EPA's Response to Comments Received on the April 28, 2016, Notice for a Pesticide Product Application with a New Active Ingredient Wolbachia pipientis, ZAP Strain in Mal Aedes albopictus (Asian Tiger Mosquito) (EPA File Symbol 89668-U) - Docket ID Number: EPA-HQ-OPP-0205; FRL-9945-49). Dated September 07, 2017.

Mains, J.W., Brelsfoard, C.L., Rose, R.I., and Dobson, S.L. (2016): Female Adult *Aedes albopictus* Suppression by *Wolbachia*-Infected Male Mosquitoes. Scientific Reports 6: 33846. - Available at: https://www.nature.com/articles/srep33846

MIT Technology Review, July 14, 2017: Verily Robot Will Raise 20 Million Sterile Mosquitoes for Release in California - Available at: https://www.technologyreview.com/s/608280/alphabet-has-built-a-robot-that-is-releasing-millions-of-sterile-mosquitoes-in-california/

USDA Plant Hardiness Zone Map - Available at: http://planthardiness.ars.usda.gov/PHZMWeb/Images/All_states_halfzones_poster_300dpi.jpg

World Mosquito Program (formerly Eliminate Dengue Program) - Available at: http://www.eliminatedengue.com/program